Genetic association study of *APOA1/C3/A4/A5* gene cluster and haplotypes on triglyceride and HDL cholesterol in a community-based population

Kuo-Liong Chien ^{a,b}, Ming-Fong Chen ^b, Hsiu-Ching Hsu ^b, Ta-Chen Su ^b,
Wei-Tien Chang ^c, Chii-Ming Lee ^b, Yuan-Teh Lee ^{b,*}

^a Institute of Preventive Medicine, School of Public Health, National Taiwan University, Taiwan

^b Department of Internal Medicine, National Taiwan University Hospital, Taipei 100, Taiwan

^c Department of Emergency Medicine, National Taiwan University Hospital, Taiwan

Received 6 October 2005; received in revised form 4 October 2007; accepted 4 October 2007

Available online 11 October 2007

Abstract

Background: Polymorphism of apolipoprotein *A1/C3/A4/A5* gene cluster affected lipid profiles in general population. We reported 6 polymorphisms, *APOA1* −75G>A, *APOA1* 83C>T, *APOC3* 3175C>G, *APOC3* 3206G>T, *APOA4* 127A>G, and *APOA5* 553G>T in *APOA1/C3/A4/A5* gene and the haplotype structures on triglyceride and HDL traits among ethnic Chinese.

Results: Overall, there were statistically significant differences in the distribution of *APOA1* −75G>A and *APOA5* +553G>T genotypes comparing cases with control subjects. For the *APOA1* −75 SNP, a lower risk of triglyceride/HDL among subjects with A/A genotype compared with those with the G/G genotype (odds ratio, OR=0.39, 95% CI 0.16–0.92, *P*=0.04). However, the risk magnitude reduced after multivariate adjustments. For continuous traits, we found that only in *APOA5* +553 T allele carriers showed a significant higher triglyceride and a significant lower HDL cholesterol level than subjects with *APOA5* +553 G/G genotypes. There were significant differences in overall haplotype frequencies between case and control subjects (*P*<0.001).

Conclusion: There is an important role of *APOA1/C3/A4/A5* gene polymorphisms and haplotypes in the development of high triglyceride/HDL ratio in Chinese.

© 2007 Published by Elsevier B.V.

Keywords: Apolipoprotein A1/C3/A4/A5; Triglyceride; HDL cholesterol; Haplotype

1. Introduction

Dyslipidemia, including high triglyceride and low high density lipoprotein (HDL) cholesterol, was associated with coronary heart disease events [1,2]. Genetic control of hypertriglyceridemia and HDL levels was considered as important components, but the susceptibility genes for variations of triglyceride and HDL levels have not been fully elucidated. Furthermore, the genetic pleiotrophic effects were demonstrated among families [3–5]. We demonstrated major gene effects on controlling triglyceride/HDL among community and hospital-based population in Taiwan [6]. Although previous extensive

genomic scanning study on triglyceride/HDL reported to linkage with chromosome 7 [7], there were inconsistent results about susceptibility gene profiles for the trait.

Individual polymorphisms of apolipoprotein gene cluster, *APOA1/C3/A4/A5* [3,8–12] are located on chromosome 11 and >10 single nucleotide polymorphisms were found in this gene cluster [13]. Previous case-control association studies showed that *APOA1* −75G>A polymorphism over the promoter site, was associated with HDL level [14,15]. In addition, *APOA1* 83C>T was also reported with HDL [16,17]. Among *APOC3* gene, 3175C>G and 3206T>G SNPs on the exon 4 were related with triglyceride level [18,19]. With regards to *APOA4* gene, SNPs on amino acid 360G>T substitution, (amino acid Glu>His), 347A>T, (amino acid Thr>Ser) and 127A>G (Glu>His) were reported with lipid levels [20,21]. Recently identified *APOA* 5553G>T

* Corresponding author. Tel.: +886 2 23123456x2830; fax: +886 2 23519736.

E-mail address: ytleee@ha.mc0.ntu.edu.tw (Y.-T. Lee).

polymorphism was associated with lipid profiles among different ethnicities [10,11,22], and was associated with dyslipidemia and atherosclerosis.

Although there were many reports about individual gene polymorphisms on triglyceride and HDL levels, data on simultaneous consideration of several polymorphisms, especially on the haplotype structures, were available [12,23–26]. Small effects of genotypes on complex traits were difficult to detect and haplotype structures should be taken into consideration. This study, based on the ethnic Chinese community, was aimed to explore the *APOA1/C3/A4/A5* cluster loci on triglyceride and HDL traits by linkage disequilibrium, haplotype structures.

2. Materials and methods

2.1. Subjects and design

We recruited family members from the Chin-Shan community, located at the northern side of Taiwan. There were a total 116 nuclear families, composed of 388 men and 435 women (average 38 y). The sampling strategy was described previously [6,27]. In brief, the probands were recruited from the only one junior high school in the community. At first, a total of 1063 students (response rate 94.6%) agreed to participate in a general health check-up after informed consent was obtained. They underwent examinations for anthropometric measures, blood pressure, and lipid profiles. Every student and first-degree relatives were asked to answer one structured questionnaire and received physical examination. They underwent examinations for anthropometric measures, blood pressure, and lipid profiles. Seven measures, including total cholesterol, triglyceride, low density lipoprotein (LDL) cholesterol, body mass index, systolic pressure, diastolic pressure, and high density lipoprotein (HDL) cholesterol were collected. We collected data from high risk students ($n=171$) and randomized sampled students ($n=197$), and their family members were invited. Detailed medical history, smoking and drinking history were taken. We also excluded significant cardiovascular morbidity, such as coronary heart disease and stroke history in the study population. The Medical Ethics Committee of National Taiwan University, College of Public Health has approved all protocols.

2.2. Lipid/lipoprotein analyses

All venous blood samples drawn after a 12-h overnight fast were immediately refrigerated and transported within 6 h to National Taiwan University Hospital. Serum samples were then stored at -70°C prior to the batch assay for levels of triglyceride and HDL. Standard enzymatic tests for triglyceride were used (Merck 14354 and 14366, Germany, respectively). HDL levels were measured in supernatants after the precipitation of specimens with magnesium chloride phosphotungstate reagents (Merck 14993) [28].

2.3. DNA preparation and genotyping

The blood was collected by venipuncture in a heparinized tube (10 U/ml) and immediately centrifuged at $150 \times g$ for 20 min at room temperature. The buffy coat was pooled, layered over Ficoll-sodium diatrizoate (Ficoll-Paque™ Plus; Amersham Bioscience, Uppsala, Sweden), and centrifuged at $250 \times g$ for 15 min at room temperature. The peripheral blood mononuclear cell (PBMC) layer was collected and genomic DNA was isolated [29].

For genotyping, the polymerase chain reaction (PCR) was used for amplification of the target regions. The primers, experimental conditions, target sizes and the allele distinction are listed. All PCRs were performed with initial denaturation of 95°C for 3 min before undergoing the 35 cycles of the indicated program. The reaction ended with an additional 7 min of the last extending temperature. The DNAs digested by restriction enzyme were separated on 2–3% agarose gel containing ethidium bromide in $1 \times$ TBE buffer and the polymorphism typed by visualization under UV light [14,30,31]. We chose the *APOA5* +553G>T SNP due to strong linkage disequilibrium with other

SNPs such as $-1131\text{T}>\text{C}$ [32], and the tagSNP analysis from HAPMAP [33] showed only one block in *APOA5* gene. *APOA5* polymorphism could lower plasma triglyceride levels by increasing lipoprotein lipase activity [34] and was considered a candidate gene for dyslipidemia and atherosclerosis [11,35].

2.4. Statistical analysis

All data were presented by mean $\pm$ SD. We collected total 444 spouses in the population, and only 156 spouse pairs were used to estimate allele frequency, haplotype patterns and linkage disequilibrium coefficients. We estimated the frequencies of the alleles by gene counting. Agreement of genotype frequencies with Hardy–Weinberg equilibrium expectations was tested using χ^2 goodness-of-fit test with one degree of freedom. Pair-wise linkage disequilibrium coefficients, including Lewontin's D' and correlation coefficients (r^2) were estimated iteratively and significance testing was calculated among the control subjects [36,37]. Haplotype frequencies were estimated by SAS/Genetics using the expectation–maximization algorithm [38]. We used the χ^2 test to examine whether overall haplotype frequencies were significantly different between case and control subjects.

Serum triglyceride and HDL cholesterol level difference among every genotype was also tested with multiple linear regression models, after adjusting for age, gender, body mass index, smoking and drinking status. We designated the inferred haplotype distribution in the study participants accounting for haplotype uncertainty by multiple imputation methods [39]. Because triglyceride/HDL ratios were the continuous variables, we adapted the case/control study design by extreme group analyses [40]. In brief, we defined the top quintile of the triglyceride/HDL values in study population as case group and the bottom quintile as control group. These subset data were used to estimate the association of genes and traits. The extreme group method was demonstrated to have high power, and especially useful in affected sibpair and linkage analysis study design [41,42]. The adjusted odds ratios were calculated by using multivariate logistic regression analysis, and we used the co-dominant modes of inheritance to avoid assumptions. We used the generalized estimation equation (GEE) model in the case/control subjects to handle the dependence problem within each family. We corrected the multiple testing problems by using Bonferroni correction, Hommel test [43] and false recovery rate [44]. We designated the inferred haplotype distribution in the study participants accounting for haplotype uncertainty by multiple imputation methods, which were implemented in the SAS Macro program, HAPPY [39].

3. Results

3.1. Descriptive data

According to the extreme grouping strategy, we included 163 control subjects and 165 case patients into this study. Characteristics of the case and control subjects are presented in Table 1. The baseline age, total and LDL cholesterol, triglyceride apolipoprotein B, body mass index, waist circumference and blood pressure were significantly higher in case than control subjects. HDL cholesterol and apolipoprotein A levels were significantly lower in case than control subjects. Case subjects were less likely to be female, yet more likely to be smokers and alcohol drinkers.

3.2. Single-locus association study in binary trait and continuous variables

We tested the association between various SNPs and triglyceride/HDL trait in the study participants. Overall, there were statistically significant differences in the distribution of *APOA1* $-75\text{G}>\text{A}$ and *APOA5* $+553\text{G}>\text{T}$ genotypes comparing cases with control subjects (Table 2). For the *APOA1* -75 SNP,

Table 1
Distribution of the anthropometric and lipid profiles in the study participants, specified by case and control status

Numbers	Case subjects		Control subjects		<i>P</i> value
	165		163		
	Mean	SD	Mean	SD	
Age (y)	47.6	17.7	29.3	17	<.0001
Total cholesterol (mg/dl)	211.5	43.1	174.0	37.9	<.0001
Triglyceride (mg/dl)	285.4	199.9	53.6	11.3	<.0001
HDL cholesterol (mg/dl)	33.0	7.6	54.1	9.3	<.0001
LDL cholesterol (mg/dl)	140.7	43.3	86.9	37.3	<.0001
Apolipoprotein A1 (mg/dl)	113.9	22.3	126.4	24.6	<.0001
Apolipoprotein B (mg/dl)	78.0	23.7	43.8	13.1	<.0001
Triglyceride/HDL ratio	9.55	9.73	1.01	0.22	<.0001
Body mass index	25.6	3.2	20.2	2.9	<.0001
Waist circumference	83.5	12.0	65.4	9.7	<.0001
Systolic blood pressure	125.8	16.4	110.0	13.6	<.0001
Diastolic blood pressure	81.4	11.4	70.0	9.6	<.0001
	(%)		(%)		
Women	40.0		59.5		0.0004
Current smoker	38.3		5.5		<.0001
Current drinker	36.2		10.0		<.0001

Abbreviations: SD; standard deviation; HDL, high density lipoprotein; LDL, low density lipoprotein.

a lower risk of triglyceride/HDL among subjects with A/A genotype compared with those with the G/G genotype (odds ratio, OR=0.39, 95% CI 0.16–0.92, $P=0.04$). However, the risk magnitude reduced after multivariate adjustments. Individual SNP at *APOA5* +553 position was significantly associated with high triglyceride/HDL risk: the OR comparing subjects with G/T+T/T genotypes with those with G/G genotype was 4.34 (95% CI, 1.97–9.57), and the risk persisted in the multivariate model. We found that only *APOA5* +553 SNP was significantly associated with high triglyceride/HDL trait, after adjusting multiple comparisons. For continuous traits, we

Table 3
Distributions of triglyceride and HDL cholesterol levels in the study participants, specified by case and control subjects

		Triglyceride			HDL cholesterol		
		Adjusted mean ^a	SEM	P	Adjusted mean ^a	SEM	P
<i>APOA1</i> −75	GG	168.2	13.2	NS	43.5	1.2	NS
	GA	140.2	12.9		43.9	1.2	
	AA	163.4	26.0		45.9	2.4	
<i>APOA1</i> +83	CC	157.5	8.8	NS	43.8	0.8	NS
	CT	101.1	41.5		48.0	3.8	
	+TT						
<i>APOC3</i> +3175	CC	158.1	11.8	NS	43.7	1.1	NS
	CG	147.6	14.0		44.6	1.3	
	GG	170.3	31.2		42.4	2.8	
<i>APOC3</i> +3206	GG	148.1	11.1	NS	44.6	1.0	NS
	GT	172.1	14.7		42.7	1.3	
	TT	121.4	39.4		45.1	3.6	
<i>APOA4</i> +127	AA	156.7	12.1	NS	44.0	1.1	NS
	AG	149.8	14.1		44.4	1.3	
	GG	167.2	27.9		42.1	2.5	
<i>APOA5</i> +553	GG	143.8	9.0	0.011	45.3	0.9	0.002
	G T	205.4	22.2		38.0	2.1	
	+TT						

Abbreviation: SEM, standard error or mean.

^a Adjusted after age, gender, body mass index, smoking and drinking status.

found that only in *APOA5* +553 T allele carriers showed a significant higher triglyceride and a significant lower HDL cholesterol level than subjects with *APOA5* +553 G/G genotypes (Table 3). No significant differences of triglycerides and HDL cholesterol levels across *APOA1/C3/A4* genotypes were found in the study participants. We found that only *APOA5* +553 gene was significantly associated with high triglyceride/HDL trait, after adjusting multiple comparisons. Other lipid profiles and metabolic syndrome components did not relate to *APOA1/C3/A4/A5* genotype.

Table 2
Association between SNPs in *APOA1/C3/A4/A5* gene and risk of high triglyceride/HDL ratio

		Case subjects		Control subjects		P value	Unadjusted OR	95% CI		Multivariate OR ^a	95% CI	
		Number	%	Number	%							
<i>APOA1</i> −75	GG	82	40.5	66	49.7	0.043	1.0			1.0		
	GA	71	44.2	72	43.0		0.68	0.41	1.15	0.55	0.21	1.47
	AA	12	15.3	25	7.3		0.39	0.16	0.92	0.56	0.12	2.52
<i>APOA1</i> +83	CC	160	94.5	154	97.0	NS	1.0			1.0		
	CT+TT	5	5.5	9	3.0		1.07	0.30	3.77	0.70	0.05	10.6
<i>APOC3</i> +3175	CC	84	56.4	92	50.9	NS	1.0			1.0		
	CG	68	38.0	62	41.2		0.91	0.55	1.51	0.80	0.31	2.07
	GG	13	5.5	9	7.9		1.67	0.61	4.55	2.56	0.42	15.7
<i>APOC3</i> +3206	GG	98	59.4	95	58.3	NS	1.0			1.0		
	GT	59	35.8	60	36.8		1.00	0.59	1.68	1.19	0.45	3.13
	TT	8	4.9	8	4.9		1.75	0.55	5.57	1.42	0.07	29.4
<i>APOA4</i> +127	AA	78	47.3	80	49.1	NS	1.0			1.0		
	AG	63	38.2	68	41.7		0.78	0.47	1.31	0.97	0.36	2.58
	GG	24	14.6	15	9.2		1.50	0.63	3.58	3.38	0.71	16.1
<i>APOA5</i> +553	GG	126	76.2	152	93.3	0.0004	1.0			1.0		
	GT+TT	39	23.8	11	6.7		4.34	1.97	9.57	9.43	1.93	46.0

Data are number (%).

^a Adjusted for age, gender, alcohol drinker, smoking, and body mass index.

Table 4

Haplotype frequency difference between case and control subjects and estimated odds ratios and 95% confidence intervals for various haplotypes, with the most frequent haplotype (Hap 1) as the reference group

Inferred haplotypes							Case subjects	Control subjects	Adjusted	95%
							(%)	(%)	odds	Confidence
									ratio ^a	Interval
Hap 1	A	C	G	T	G	G	25	21	1.0	
									(reference)	
Hap 2	G	C	G	T	G	G	14	21	0.60	0.22 1.67
Hap 3	A	T	G	G	G	T	15	11	0.81	0.27 2.44
Hap 4	A	T	C	G	A	T	12	13	1.98	0.58 6.75
Hap 5	A	T	C	T	A	T	10	14	0.77	0.27 2.16
Hap 6	A	C	G	T	G	T	7	5	3.88	0.56 27.0
All							17	15	0.86	0.38 1.95
others										

Likelihood ratio tests between models with and without haplotypes: $-2 \text{ Log-likelihood} = 101.7$ ($df=6$), $P<0.001$.

^a Adjusted after age, gender, body mass index, smoking and drinking status.

3.3. Hardy–Weinberg equilibrium among control subjects, haplotype frequencies and association study

All genotypes among unrelated spouse individuals were compatible with Hardy–Weinberg equilibrium. And among 64 possible haplotype combinations of the 6 genes, we found that there was significant linkage disequilibrium (chi-square = 678.82, $df=26$, $P<0.00001$). Table 4 presented the frequencies of the six common haplotypes defined by the 6 SNPs for all case and control subjects. Overall, there were significant differences in overall haplotype frequencies between case and control subjects ($P<0.001$). However, with reference to the most frequent haplotype as the baseline, the adjusted odds ratios of various haplotypes did not reach significant levels.

4. Discussion

Our data suggested an important role of *APOA1/C3/A4/A5* gene polymorphisms and haplotypes in the development of high triglyceride/HDL ratio in Chinese. This gene cluster was in strong linkage disequilibrium and most prevalent haplotypes were found. Among them, *APOA5* polymorphism was strongly associated with high triglyceride and low HDL cholesterol levels. To the authors' knowledge, this is the first report on the general community-based population of ethnical Chinese.

Variations of *APOA5* gene, located 30 kbp downstream of the *APOA1/C3/A4* gene cluster, were associated with familial combined hyperlipidemia [25], triglyceride levels [22,45], and coronary artery disease risk [35]. Using a case-control design, Tang et al. found that the *APOA5* +553C>T was associated with hypertriglyceridemia and coronary heart disease among ethnic Chinese [10]. Haplotypes with *APOA5* variation were associated with hypertriglyceridemia in different populations [23,46]. The *APOA1/C3/A4/A5* gene cluster attributed appreciably to dyslipidemia and atherosclerotic risk [12,26,47]. Our findings demonstrated that *APOA5* variant was a significant predictor for high triglyceride/HDL risk and the *APOA1/C3/A4/A5* haplotypes affected dyslipidemia appreciably among ethnic Chinese.

Previous case-control studies demonstrated that hyperlipidemic patients had significantly different allele frequency than healthy controls [17,30,48,49]. The mechanisms were assumed to be due to alterations of apolipoprotein clusters, and variation of *APOA1/C3/A4/A5* intragenic polymorphism was reported to affect expression of special genes [50]. Also, to have effective metabolism and remodeling of TG/HDL lipoproteins, a number of different apolipoprotein and functionally effective LDL receptor-related protein and LDL receptors in addition to *APOA1/C3/A4/A5* are involved in the process of triglyceride and HDL metabolism. Our study clearly demonstrated that polymorphisms in *APOA1/C3/A4/A5* gene cluster played important roles for controlling triglyceride and HDL traits. Our data indicate an important role for the polymorphism in gene clusters in controlling serum triglyceride and HDL levels. Although previous data have associated the *APOC3* locus with extremely high plasma triglyceride levels in humans [18], our study indicated that other sites, such as *APOA1* genomic interval also represented independent influence on triglyceride in humans. These results suggest the possible use of these gene cluster polymorphisms as prognostic indicators for susceptibility of dyslipidemia, and gene modulation can be used as a potential strategy to reduce cardiovascular disease risks.

There exists significant racial variation of allele frequencies in this gene cluster. Although there were reports about other novel polymorphisms of *APOA4* genes related to lipid metabolism, we have analyzed amino acid 360 (G to T substitution, Glu to His) and amino acid 347 (A to T, Thr to Ser) but the results showed no substitution was found in our study population. Our study results were compatible with Korean data, which also demonstrated linkage disequilibrium of *APOA1/C3/A4* gene cluster and associated with lipid profiles [51].

Several issues in this study were mentioned. First, we used only a subset of study subjects to apply the extreme group methods. We tested the odds ratios, with intentionally identifying the gene controlling extreme traits, not the gradual mean values, which were estimated from all subjects by general linear model. We found that the patterns and trends were similar by the general linear model in all subjects. The simulation results showed that the power of extreme group method had more

power than the general linear regression model [52]. Second, we did not consider extensive gene and environmental interactions for controlling dyslipidemia. Dietary factors and physical activity were not controlled in this study, although we controlled the dependency among family members. Dyslipidemia was a complex trait, and it is a difficult task to estimate significant main effect of one genotype, due to limited sample size and mild to moderate genetic effects.

There were study limitations in the study. First, the *APOA1* 83C>T polymorphism was too rare to add more information. Second, we restricted relatively young probands and family members, which did not include diseased subjects. Gene-environmental interactions might be more complicated among adult diseased population. Our homogenous population may be helpful to identify the genomic effects on complex trait. Third, multiple statistical testing in one-locus model was performed and it would inflate type I error.

Acknowledgements

We thank the participants in the Chin-Shan community and the cardiologists at National Taiwan University Hospital for their assistance in this study. This study was supported partly by grants from the National Science Council in Taiwan (NSC 93-2314-B-002-218, NSC 94-2314-B-002-290) and National Taiwan University Hospital (NTUH.92S025).

References

- [1] Jeppesen J, Hein HO, Suadicani P, Gyntelberg F. Triglyceride concentration and ischemic heart disease: an eight-year follow-up in the Copenhagen Male Study. *Circulation* 1998;97:1029–36.
- [2] Assmann G, Schulte H. Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience). Prospective Cardiovascular Munster study. *Am J Cardiol* 1992;70:733–7.
- [3] Mahaney MC, Blangero J, Rainwater DL, et al. A major locus influencing plasma high-density lipoprotein cholesterol levels in the San Antonio Family Heart Study. Segregation and linkage analyses. *Arterioscler Thromb Vasc Biol* 1995;15:1730–9.
- [4] Edwards KL, Mahaney MC, Motulsky AG, Austin MA. Pleiotropic genetic effects on LDL size, plasma triglyceride, and HDL cholesterol in families. *Arterioscler Thromb Vasc Biol* 1999;19:2456–64.
- [5] Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 1990;82:495–506.
- [6] Chien KL, Hsu HC, Su TC, Yang CY, Lee YT. Consistency of genetic inheritance mode and heritability patterns of triglyceride vs. high density lipoprotein cholesterol ratio in two Taiwanese family samples. *BMC Genet* 2003;4:7.
- [7] Shearman AM, Ordovas JM, Cupples LA, et al. Evidence for a gene influencing the TG/HDL-C ratio on chromosome 7q32.3-qter: a genome-wide scan in the Framingham study. *Hum Mol Genet* 2000;9:1315–20.
- [8] Juo SH, Beaty TH, Duffy DL, Coresh J, Kwiterovich Jr PO. No common major gene for apolipoprotein A-I and HDL3-C levels: evidence from bivariate segregation analysis. *Genet Epidemiol* 1999;16:54–68.
- [9] Prenger VL, Beaty TH, Kwiterovich PO. Genetic determination of high-density lipoprotein-cholesterol and apolipoprotein A-I plasma levels in a family study of cardiac catheterization patients. *Am J Hum Genet* 1992;51:1047–57.
- [10] Tang Y, Sun P, Guo D, et al. A genetic variant c.553G>T in the apolipoprotein A5 gene is associated with an increased risk of coronary artery disease and altered triglyceride levels in a Chinese population. *Atherosclerosis* 2006;185:433–7.
- [11] Wright WT, Young IS, Nicholls DP, Patterson C, Lyttle K, Graham CA. SNPs at the APOA5 gene account for the strong association with hypertriglyceridaemia at the APOA5/A4/C3/A1 locus on chromosome 11q23 in the Northern Irish population. *Atherosclerosis* 2006;185:353–60.
- [12] Mar R, Pajukanta P, Allayee H, et al. Association of the APOLIPOPROTEIN A1/C3/A4/A5 gene cluster with triglyceride levels and LDL particle size in familial combined hyperlipidemia. *Circ Res* 2004;94:993–9.
- [13] Humphries SE. DNA polymorphisms of the apolipoprotein genes—their use in the investigation of the genetic component of hyperlipidaemia and atherosclerosis. *Atherosclerosis* 1988;72:89–108.
- [14] Kamboh MI, Aston CE, Nestlerode CM, McAllister AE, Hamman RF. Haplotype analysis of two APOA1/MspI polymorphisms in relation to plasma levels of apo A-I and HDL-cholesterol. *Atherosclerosis* 1996;127:255–62.
- [15] Civeira F, Pocovi M, Cenarro A, Garces C, Ordovas JM. Adenine for guanine substitution-78 base pairs 5' to the apolipoprotein (APO) A-I gene: relation with high density lipoprotein cholesterol and APO A-I concentrations. *Clin Genet* 1993;44:307–12.
- [16] Larson IA, Ordovas JM, Barnard JR, et al. Effects of apolipoprotein A-I genetic variations on plasma apolipoprotein, serum lipoprotein and glucose levels. *Clin Genet* 2002;61:176–84.
- [17] Pulkkinen A, Viitanen L, Kareinen A, Lehto S, Laakso M. MspI polymorphism at +83 bp in intron 1 of the human apolipoprotein A1 gene is associated with elevated levels of HDL cholesterol and apolipoprotein A1 in nondiabetic subjects but not in type 2 diabetic patients with coronary heart disease. *Diabetes Care* 2000;23:791–5.
- [18] Tsai MY, Hanson NQ, Copeland KR, Beheshti I, Garg U. Determination of a T/G polymorphism at nucleotide 3206 of the apolipoprotein C III gene by amplification refractory mutation system. *Clin Chem* 1994;40:2235–9.
- [19] Hegele RA, Connelly PW, Hanley AJ, Sun F, Harris SB, Zinman B. Common genomic variation in the APOC3 promoter associated with variation in plasma lipoproteins. *Arterioscler Thromb Vasc Biol* 1997;17:2753–8.
- [20] Kamboh MI, Friedlaender JS, Ahn YI, Ferrell RE. A common deletion polymorphism in the apolipoprotein A4 gene and its significance in lipid metabolism. *Arterioscler Thromb* 1994;14:656–62.
- [21] Saha N, Wang G, Vasisht S, Kamboh MI. Influence of two apo A4 polymorphisms at codons 347 and 360 on non-fasting plasma lipoprotein-lipids and apolipoproteins in Asian Indians. *Atherosclerosis* 1997;131:249–55.
- [22] Hodoglugil U, Tanyolac S, Williamson DW, Huang Y, Mahley RW. Apolipoprotein A-V: a potential modulator of plasma triglyceride levels in Turks. *J Lipid Res* 2006;47:144–53.
- [23] Kao JT, Wen HC, Chien KL, Hsu HC, Lin SW. A novel genetic variant in the apolipoprotein A5 gene is associated with hypertriglyceridemia. *Hum Mol Genet* 2003;12:2533–9.
- [24] Dallinga-Thie GM, van Linde-Sibenius Trip M, Rotter JJ, et al. Complex genetic contribution of the Apo A1–CIII–AIV gene cluster to familial combined hyperlipidemia. Identification of different susceptibility haplotypes. *J Clin Invest* 1997;99:953–61.
- [25] Eichenbaum-Voline S, Olivier M, Jones EL, et al. Linkage and association between distinct variants of the APOA1/C3/A4/A5 gene cluster and familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 2004;24:167–74.
- [26] Talmud PJ, Hawe E, Martin S, et al. Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides. *Hum Mol Genet* 2002;11:3039–46.
- [27] Chien KL, Chen WJ, Hsu HC, Su TC, Chen MF, Lee YT. Major gene effects on apolipoprotein B concentrations in families of adolescents—results from a community-based study in Taiwan. *Clin Chim Acta* 2006;365:194–9.
- [28] Warnick GR, Benderson J, Albers JJ. Dextran sulfate–Mg²⁺ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin Chem* 1982;28:1379–88.
- [29] Bell GI, Karam JH, Rutter WJ. Polymorphic DNA region adjacent to the 5' end of the human insulin gene. *Proc Natl Acad Sci U S A* 1981;78:5759–63.
- [30] Ordovas JM, Civeira F, Genest Jr J, et al. Restriction fragment length polymorphisms of the apolipoprotein A-I, C-III, A-IV gene locus. Relationships with lipids, apolipoproteins, and premature coronary artery disease. *Atherosclerosis* 1991;87:75–86.

- [31] Tenkanen H, Koskinen P, Metso J, et al. A novel polymorphism of apolipoprotein A-IV is the result of an asparagine to serine substitution at residue 127. *Biochim Biophys Acta* 1992;1138:27–33.
- [32] Matsunaga A, Arishima H, Niimura H, et al. Strong linkage disequilibrium and association of -1131T>C and c.553G>T polymorphisms of the apolipoprotein A5 gene with hypertriglyceridemia in a Japanese population. *Circ J* 2007;71:746–52.
- [33] A haplotype map of the human genome. *Nature* 2005;437:1299–320.
- [34] Merkel M, Loeffler B, Kluger M, et al. Apolipoprotein AV accelerates plasma hydrolysis of triglyceride-rich lipoproteins by interaction with proteoglycan-bound lipoprotein lipase. *J Biol Chem* 2005;280:21553–60.
- [35] Szalai C, Keszey M, Duba J, et al. Polymorphism in the promoter region of the apolipoprotein A5 gene is associated with an increased susceptibility for coronary artery disease. *Atherosclerosis* 2004;173:109–14.
- [36] Lewontin RC. On measures of gametic disequilibrium. *Genetics* 1988;120:849–52.
- [37] Devlin B, Risch N. A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics* 1995;29:311–22.
- [38] Zhao JH, Curtis D, Sham PC. Model-free analysis and permutation tests for allelic associations. *Hum Hered* 2000;50:133–9.
- [39] Kraft P, Cox DG, Paynter RA, Hunter D, De VI. Accounting for haplotype uncertainty in matched association studies: a comparison of simple and flexible techniques. *Genet Epidemiol* 2005;28:261–72.
- [40] Barrett J. Association study. In: Camp N, Cox A, editors. *Quantitative trait loci: methods and protocols*. Totowa, NJ: Humana Press Inc. 2002. p. 3–12.
- [41] Risch NJ, Zhang H. Mapping quantitative trait loci with extreme discordant sib pairs: sampling considerations. *Am J Hum Genet* 1996;58:836–43.
- [42] Schork NJ, Nath SK, Fallin D, Chakravarti A. Linkage disequilibrium analysis of biallelic DNA markers, human quantitative trait loci, and threshold-defined case and control subjects. *Am J Hum Genet* 2000;67:1208–18.
- [43] Hommel G. A comparison of two modified Bonferroni procedures. *Biometrika* 1988;75:383–6.
- [44] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 1995;57:289–300.
- [45] Hubacek JA. Apolipoprotein A5 and triglyceridemia. Focus on the effects of the common variants. *Clin Chem Lab Med* 2005;43:897–902.
- [46] Olivier M, Wang X, Cole R, et al. Haplotype analysis of the apolipoprotein gene cluster on human chromosome 11. *Genomics* 2004;83:912–23.
- [47] Lai CQ, Parnell LD, Ordovas JM. The APOA1/C3/A4/A5 gene cluster, lipid metabolism and cardiovascular disease risk. *Curr Opin Lipidol* 2005;16:153–66.
- [48] Kessling AM, Rajput-Wiliams J, Bainton D, et al. DNA polymorphisms of the apolipoprotein AII and AI-CIII-AIV genes: a study in men selected for differences in high-density-lipoprotein cholesterol concentration. *Am J Hum Genet* 1988;42:458–67.
- [49] Wang XL, Badenhop R, Humphrey KE, Wilcken DE. New MspI polymorphism at +83 bp of the human apolipoprotein AI gene: association with increased circulating high density lipoprotein cholesterol levels. *Genet Epidemiol* 1996;13:1–10.
- [50] Walsh A, Azrolan N, Wang K, Marcigliano A, O'Connell A, Breslow JL. Intestinal expression of the human apoA-I gene in transgenic mice is controlled by a DNA region 3' to the gene in the promoter of the adjacent convergently transcribed apoC-III gene. *J Lipid Res* 1993;34:617–23.
- [51] Song J, Park JW, Park H, Kim JQ. Linkage disequilibrium of the Apo AI-CIII-AIV gene cluster and their relationship to plasma triglyceride, apolipoprotein AI and CIII levels in Koreans. *Mol Cells* 1998;8:12–8.
- [52] Tregouet DA, Ducimetiere P, Tiret L. Testing association between candidate-gene markers and phenotype in related individuals, by use of estimating equations. *Am J Hum Genet* 1997;61:189–99.